HPLC FINGERPRINT SPECTRUM ANALYSIS OF ALKALOIDS IN ARECAE SEMEN

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***Abstract**: Arecae Semen, the dried ripe seed of Areca catechu L. (Arecaceae), is an important medicinal material widely used in traditional medicine throughout Vietnam and other Asian countries. Alkaloids are recognized as the characteristic bioactive constituents of Arecae Semen and played a crucial role in its quality evaluation. However, variations in geographical origin and harvest maturity may result in considerable differences in alkaloid composition, leading to inconsistency in the quality of commercial materials. To establish and validate a high-performance liquid chromatography (HPLC) fingerprint method based on characteristic alkaloid constituents for the comprehensive quality evaluation of Arecae Semen samples collected from different cultivation regions in Vietnam. Thirty batches of Arecae Semen samples were collected from major cultivation areas across Vietnam. Chromatographic separation was achieved on a BEH Amide column using gradient elution with acetonitrile and 0.2% phosphoric acid containing 0.2% triethylamine, and UV detection was performed at 210 nm. Four characteristic alkaloids, namely arecoline, guvacoline, arecaidine and guvacine were employed as reference compounds for peak assignment and fingerprint characterization. Similarity analysis was subsequently applied to assess chemical consistency among samples. The validated method demonstrated satisfactory precision, repeatability, and stability, with retention-time RSD values below 3.0%. Most ripe Arecae Semen samples exhibited high fingerprint similarity values (> 0.90), indicating consistent alkaloid profiles, whereas immature samples showed significantly lower similarity values, reflecting substantial variations in alkaloid composition. The combination of chromatographic fingerprinting with four reference alkaloids offers an effective strategy for quality control and may provide a scientific basis for the establishment of future pharmacopoeial standards for Arecae Semen.*

***Keywords:** Arecae Semen; Areca catechu L.; alkaloids; HPLC fingerprint; arecoline.*

1. INTRODUCTION

Arecae Semen, the dried ripe seed of *Areca catechu* L. (Arecaceae) [1 – 3], is a traditional medicinal material extensively used in many Asian countries, including Vietnam. In traditional medicine, it is commonly prescribed for promoting digestion, relieving food retention, regulating gastrointestinal function, and expelling intestinal parasites [1, 2]. Owing to its widespread medicinal use and increasing commercial demand, the establishment of reliable quality evaluation methods for Arecae Semen has become essential for ensuring its safety, efficacy, and consistency.

Phytochemical investigations have demonstrated that Arecae Semen contains a variety of chemical constituents, including alkaloids, tannins, polyphenols, amino acids, polysaccharides, and fatty acids. Among these constituents, alkaloids are considered the characteristic and pharmacologically relevant compounds of the herb. Previous studies have identified guvacoline, arecoline, guvacine, and arecaidine as the major alkaloids in Arecae Semen, and these compounds have been widely recognized as important markers for quality assessment and standardization [4, 5].

Vietnam is one of the major cultivation areas of *A. catechu* in Southeast Asia, with areca palms distributed across diverse ecological regions from northern to southern provinces. Nevertheless, environmental conditions, geographical origin, cultivation practices, and harvest maturity may significantly influence alkaloid biosynthesis and accumulation, resulting in variations in the chemical quality of Arecae Semen samples. Despite the widespread cultivation and utilization of areca in Vietnam, systematic

investigations on the chemical consistency of Arecae Semen collected from different regions remain limited.

Current quality standards for Arecae Semen mainly rely on the determination of individual marker compounds, particularly arecoline. Although single-component analysis is suitable for routine quality control, it cannot adequately reflect the overall chemical characteristics and compositional relationships among alkaloid constituents. Chromatographic fingerprint analysis has therefore emerged as a powerful approach for the holistic evaluation of herbal medicines, allowing simultaneous characterization of multiple compounds and assessment of their relative distribution patterns.

In the present study, an HPLC fingerprint method focusing on alkaloid constituents was established and validated for the quality evaluation of Arecae Semen samples collected from representative cultivation regions throughout Vietnam. Four characteristic alkaloids, namely guvacoline, arecoline, guvacine, and arecaidine, were used as reference compounds for peak identification and fingerprint characterization. The established fingerprint was subsequently applied to evaluate the chemical consistency of different sample batches and to explore the influence of harvest maturity on alkaloid profiles. The results provide scientific evidence for quality control, standardization, and the future development of pharmacopoeial standards for Arecae Semen in Vietnam.

Therefore, the present study aimed to develop and validate an alkaloid-based HPLC fingerprint method for Arecae Semen using four characteristic alkaloid constituents, namely arecoline, guvacoline, arecaidine, and guvacine. The established fingerprint was further applied to evaluate the chemical consistency of Arecae Semen samples collected from different regions of Vietnam and to investigate its potential for distinguishing mature and immature samples. The ultimate goal was to provide a scientific basis for improving the quality control and standardization of Arecae Semen.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

2.1.1. Instruments

Agilent 1260 HPLC; ML204 electronic analytical balance (Mettler-Toledo Instruments Co., Ltd., Shanghai).

1.1.2. Reference Standards and Chemicals

- Reference standards: Information on reference standards is provided in Table 1.

***Table 1**. Information on Reference Standards*

Name	Origin	Lot number	Content
Arecoline hydrobromide	National Institutes for Food and Drug Control, China	111684-202003	99.8%
Guvacoline	Chengdu Dest Bio-technology Co., Ltd, China	DST240906-178	98%
Arecaidine	Chengdu Dest Bio-technology Co., Ltd, China	DST220716-178	98%
Guvacine	Chengdu Dest Bio-technology Co., Ltd, China	DSTDQ021801	98%

Chemicals: Acetonitrile was of chromatographic grade, and all other reagents were of analytical grade.

2.1.3. Samples

A total of 30 batches of Arecae Semen samples were included in this study. Samples coded VNA1–VNA10 were collected from ripe fruits of *Areca catechu* L. with green pericarps at the harvesting stage. Samples coded VNA11 – VNA15 were commercially available materials purchased from local herbal markets. Samples coded VNA16 – VNA30 were collected from fully ripe fruits characterized by yellow or red pericarps.

For freshly collected samples, the fruits were manually peeled, and the seeds were separated and dried in a forced-air drying oven at 42°C until the moisture content was reduced to below 10%. The dried seeds were subsequently pulverized and passed through a No. 5 sieve to obtain a homogeneous powder for chemical analysis.

All samples were authenticated as Arecae Semen, the dried mature seeds of *Areca catechu* L. (Arecaceae), by Huang Qingquan, Deputy Chief Technician, Institute of Traditional Chinese Medicine, Guangxi Institute for Drug Control, Nanning, China.

Detailed information on the sample sources and collection locations is presented in Table 2.

Table 2. The list of Samples

No	Name	Batch	Origin	No	Name	Batch	Origin
1	Arecae Semen	VNA1	Hung Yen	16	Arecae Semen	VNA16	Nam Dinh
2	Arecae Semen	VNA2	Hung Yen	17	Arecae Semen	VNA17	Ha Noi
3	Arecae Semen	VNA3	Hung Yen	18	Arecae Semen	VNA18	Ha Noi
4	Arecae Semen	VNA4	Dong Nai	19	Arecae Semen	VNA19	Hai Phong
5	Arecae Semen	VNA5	Quang Nam	20	Arecae Semen	VNA20	Hai Phong
6	Arecae Semen	VNA6	Quang Nam	21	Arecae Semen	VNA21	Hai Phong
7	Arecae Semen	VNA7	Quang Nam	22	Arecae Semen	VNA22	Hai Phong
8	Arecae Semen	VNA8	Dak Lak	23	Arecae Semen	VNA23	Thanh Hoa
9	Arecae Semen	VNA9	Ha Tinh	24	Arecae Semen	VNA24	Thanh Hoa
10	Arecae Semen	VNA10	Nghe An	25	Arecae Semen	VNA25	Quang Ngai
11	Arecae Semen	VNA11	Vietnam	26	Arecae Semen	VNA26	Binh Duong
12	Arecae Semen	VNA12	Vietnam	27	Arecae Semen	VNA27	Dak Lak
13	Arecae Semen	VNA13	Vietnam	28	Arecae Semen	VNA28	Dong Nai
14	Arecae Semen	VNA14	Vietnam	29	Arecae Semen	VNA29	Dong Nai
15	Arecae Semen	VNA15	Vietnam	30	Arecae Semen	VNA30	Dong Nai

Chromatography condition

The filler was Waters XBridge BEH Amide column (4.6×250 mm, 5 µm). The mobile phase and detection wavelength were chosen after referring to many articles and comparison. The final mobile phase was A (acetonitrile) and B (0.2% phosphoric acid (containing 0.2% triethylamine)). The detection wavelength was 210 nm, the column temperature was 30°C, the flow rate was 1 mL/min. The gradient elution conditions are shown in Table 3.

Table 3. The conditions of gradient elution

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0 ~ 20	81	19
20 ~ 40	81→70	19→30

1.2. Preparation of standard and Sample solutions

2.3.1. Preparation of reference standard

Mixed reference standard solution: Appropriate amounts of arecoline hydrobromide, guvacoline, arecaidine, and guvacine reference standards were accurately weighed and dissolved in methanol to obtain a mixed standard solution containing 10 µg/mL of arecoline hydrobromide (equivalent arecoline concentration = arecoline hydrobromide/1.5214), 15 µg/mL of guvacoline, 30 µg/mL of arecaidine, and 15 µg/mL of guvacine.

2.3.2. Preparation of sample solution

Weigh accurately 0.5 g of the powder (passed through No. 5 sieve), moistened with 2 mL of ammonia solution for 5 minutes, add accurately 20 mL of methanol and weigh. Ultrasonicate for 30 minutes (power 500 W, frequency 40 kHz, at room temperature), cool and weigh again, replenish the loss of solvent with methanol, mix well and filter. Evaporate accurately 10 mL of the subsequent filtrate to near dryness at reduced pressure in a rotary evaporator. Dissolve the residue in accurately10 mL of 50% acetonitrile (containing 0.1% phosphoric acid), mix well and filter, use the subsequent filtrate as the test solution.

Chromatographic conditions and sample preparation procedures were optimized based on comparative experiments involving extraction method, extraction time and sample amount. Detailed optimization data are provided in Supplementary Materials [8].

1.3. Establishment of Alkaloid Fingerprint [3, 6, 7]

Based on the results of alkaloid content determination, immature samples (VNA1 - VNA10) were excluded from the construction of the reference fingerprint chromatogram. Fifteen representative batches collected from different cultivation regions of Vietnam (VNA16 - VNA30) were selected to establish the reference fingerprint.

The chromatographic data were imported into the Similarity Evaluation System for ***Chromatographic***

Fingerprints of Traditional Chinese Medicine (Version 2012A) to generate the reference alkaloid fingerprint chromatogram of *Arecae Semen* (Figure 1). A total of six common peaks were observed in the fingerprint profile.

Among these peaks, four characteristic peaks were identified by comparison with authentic reference standards. Peak 1, Peak 2, Peak 3, and Peak 4 were assigned as arecoline, guvacoline, arecaidine, and guvacine, respectively. These four alkaloids were selected as reference markers for fingerprint characterization and similarity evaluation of *Arecae Semen* samples.

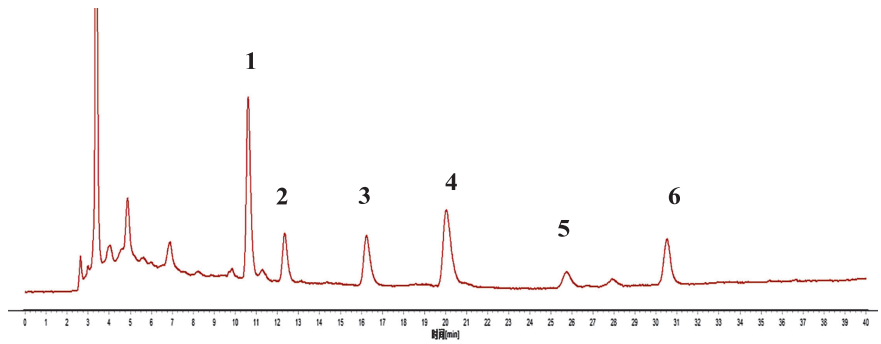

Figure 2. *Reference HPLC fingerprint chromatogram of alkaloid in Arecae Semen.*
Peak 1: Arecoline; Peak 2: Guvacoline; Peak 3: Arecaidine; Peak 4: Guvacine.

2.5. Validation method

2.5.1. Repeatability test

Sample VNA29 was selected for the repeatability test. Six test solutions were independently prepared according to the procedure described in Section 2.2 – 2.3 and analyzed under the chromatographic conditions described in Section 2.1. The retention time of the six characteristic peaks were recorded.

Table 4*. Repeatability test results of the alkaloid fingerprint method for Arecae Semen*

No	Retention time (min)					
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**
1	10.675	12.486	16.570	20.657	26.314	31.525
2	10.662	12.421	16.526	20.631	26.334	31.454
3	10.682	12.462	16.542	20.619	26.348	31.622
4	10.649	12.448	16.555	20.624	26.167	31.509
5	10.602	12.494	16.526	20.664	26.248	31.483
6	10.618	12.483	16.584	20.675	26.339	31.563
Average	10.648	12.466	16.551	20.645	26.292	31.526
RSD (%)	0.30	0.22	0.14	0.11	0.27	0.19

The results showed that the relative standard deviation (RSD) values of the retention time for all characteristic peaks were below 3.0%, indicating good repeatability of the established fingerprint method.

The low RSD values obtained for all characteristic peaks demonstrated excellent repeatability and reliability of the proposed HPLC fingerprint method.

2.5.2. Precision test

The same test solution prepared from sample VNA29 was injected six consecutive times. The RSD values of retention time for all characteristic peaks were below 3.0%, demonstrating satisfactory instrumental precision (Table 5).

Table 5. Precision test results of the alkaloid fingerprint method in Arecae Semen

No	Retention time (min)					
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
1	10.675	12.486	16.570	20.657	26.314	31.525
2	10.677	12.446	16.582	20.631	26.302	31.528
3	10.689	12.462	16.593	20.682	26.208	31.561
4	10.661	12.497	16.521	20.635	26.339	31.508
5	10.692	12.502	16.533	20.643	26.292	31.511
6	10.654	12.437	16.536	20.667	26.284	31.509
Average	10.675	12.472	16.556	20.653	26.290	31.524
RSD (%)	0.14	0.22	0.18	0.10	0.17	0.06

2.5.3. Stability test

The test solution prepared from VNA29 was analyzed at 0 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h under the specified chromatographic conditions. The characteristic chromatograms were recorded, and the retention time of the characteristic peaks were calculated. The results showed that the %RSD of the retention time of all characteristic peaks were less than 3.0%, indicating that the test solution was stable within 24 h.

Table 6. Stability test results of the alkaloid fingerprint method in Arecae Semen

No	Retention time (min)					
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
4 h	10.675	12.486	16.570	20.657	26.314	31.525
8 h	10.635	12.437	16.501	20.549	26.306	31.576
12 h	10.558	12.395	16.497	20.583	26.216	31.663
16 h	10.634	12.647	16.524	20.663	26.246	31.427
20 h	10.637	12.309	16.364	20.592	26.319	31.563
24 h	10.601	12.422	16.483	20.625	26.331	31.541
Average	10.623	12.449	16.490	20.612	26.289	31.549
RSD (%)	0.37	0.91	0.42	0.22	0.18	0.24

2.6. Statistical Analysis

All experiments were performed in triplicate unless otherwise specified. Results were expressed as mean values and relative standard deviations (RSDs). The RSD values of retention times were used to evaluate the precision, repeatability, and stability of the proposed method. Similarity analysis of chromatographic fingerprints was performed using the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2012A). Chromatographic data acquisition and processing were carried out using Agilent ChemStation software.

3. RESULTS AND DISCUSSION

3.1. Similarity evaluation of the alkaloid fingerprint chromatograms of Arecae Semen

A total of 30 batches of Arecae Semen samples were analyzed according to the chromatographic conditions described in Section 2.2. The chromatograms obtained were recorded, and all analytical data were imported into the Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System. The fingerprint chromatograms of the samples were compared with the reference fingerprint generated according to Section 2.2 – 2.3, and the similarity values were subsequently calculated. The detailed results are presented in Table 10.

The results showed that the similarity values of the fingerprint chromatograms for the 30 batches ranged from 0.604 to 1.000. Among them, 20 batches, all of which were ripe Arecae Semen samples, exhibited similarity values above 0.900, indicating a high degree of overall consistency. These findings suggest that the distribution patterns of alkaloid constituents in mature Arecae Semen are relatively uniform and that the quality of the material is comparatively stable.

In contrast, 10 immature samples (No. VNA1 – VNA10) showed similarity values below 0.900. Furthermore, substantial variation was observed among these batches, with similarity values ranging from 0.604 to 0.822, corresponding to a maximum difference of 0.218. This marked variability stands in sharp contrast to the high consistency observed among mature samples and indicates significant compositional differences from the remaining batches.

These results demonstrate that the alkaloid fingerprint chromatographic method established in this study can effectively distinguish mature from immature Arecae Semen and accurately characterize the overall differences in alkaloid composition associated with different stages of maturity.

The method exhibits good specificity and strong practical applicability and is therefore recommended for inclusion in the draft quality standard for Arecae Semen, providing reliable technical support for its quality control and quality assessment.

***Table** 7. Determination results of the alkaloid fingerprint chromatograms of Arecae Semen*

No	Batch	Similarity	No	Batch	Similarity
1	VNA1	**0.822**	16	VNA16	0.946
2	VNA2	**0.758**	17	VNA17	0.990
3	VNA3	**0.807**	18	VNA18	0.991
4	VNA4	**0.604**	19	VNA19	0.974
5	VNA5	**0.710**	20	VNA20	0.984
6	VNA6	**0.638**	21	VNA21	0.993
7	VNA7	**0.763**	22	VNA22	0.998
8	VNA8	**0.634**	23	VNA23	0.991
9	VNA9	**0.738**	24	VNA24	0.982
10	VNA10	**0.697**	25	VNA25	0.977
11	VNA11	0.959	26	VNA26	0.990
12	VNA12	0.996	27	VNA27	0.983
13	VNA13	0.990	28	VNA28	0.988
14	VNA14	0.987	29	VNA29	0.986
15	VNA15	0.994	30	VNA30	0.910

Based on the current dataset, a fingerprint similarity value of 0.90 is proposed as a preliminary acceptance criterion for ripe Arecae Semen. Further validation using a larger number of samples from different geographical origins is recommended.

4. DISCUSSION

Alkaloids are recognized as the characteristic constituents of Arecae Semen and are widely regarded as important indicators for its quality evaluation. Among them, arecoline, guvacoline, arecaidine, and guvacine have been reported as the major alkaloids and are closely associated with the pharmacological activities and quality characteristics of the medicinal material. Therefore, the establishment of a chromatographic fingerprint based on alkaloid constituents represents a rational and targeted approach for the comprehensive quality assessment of Arecae Semen.

In the present study, a hydrophilic interaction chromatography (HILIC)-based HPLC method employing a BEH Amide column was successfully established for fingerprint analysis. Compared with conventional reversed-phase

chromatographic systems, the selected chromatographic conditions provided satisfactory retention and separation of highly polar alkaloids, resulting in a stable fingerprint profile with six common peaks. Four of these peaks were identified as arecoline, guvacoline, arecaidine, and guvacine through comparison with authentic reference standards. These compounds were selected as characteristic markers because they represent the major alkaloid constituents of Arecae Semen and can effectively reflect the chemical characteristics of the herbal material.

Sample preparation is a critical factor affecting fingerprint quality and analytical reliability. The results demonstrated that extraction efficiency was significantly influenced by extraction solvent, extraction technique, extraction time, and sample amount. Among the eight extraction procedures investigated, the combination of ammonia pretreatment and ultrasonic extraction with methanol provided the highest extraction efficiency for all four alkaloids. Ammonia pretreatment may facilitate the conversion of alkaloid salts into their free-base forms, thereby improving their solubility in organic solvents and enhancing extraction efficiency. In contrast, ether-based extraction methods produced lower yields for several alkaloids, particularly arecaidine and guvacine, suggesting incomplete recovery of these relatively polar constituents. Optimization studies further showed that 30 min of ultrasonic extraction was sufficient to achieve efficient extraction, while prolonging extraction time to 60 min did not result in a significant increase in alkaloid content. Similarly, a sample amount of 0.5 g provided the most suitable balance between extraction efficiency and analytical reproducibility.

Method validation demonstrated satisfactory repeatability, precision, and stability, with all retention-time RSD values below 3.0%. These results indicate that the established fingerprint method possesses adequate analytical performance for routine quality evaluation. The low variability observed among replicate analyses also confirms the robustness of the chromatographic system and sample preparation procedure.

Fingerprint similarity evaluation revealed distinct differences between mature and immature Arecae Semen samples. Most mature samples exhibited similarity values higher than 0.90, indicating relatively consistent alkaloid distribution patterns despite differences in geographical origin. In contrast, immature samples showed substantially lower similarity values and greater inter-batch variability. These findings suggest that alkaloid biosynthesis and accumulation continue during fruit maturation and these findings suggest that harvest maturity may be one of the major factors influencing alkaloid composition and overall fingerprint characteristics of Arecae Semen. Similar observations have been reported for other medicinal plant materials, where developmental stage significantly affects the accumulation of bioactive constituents and consequently influences quality consistency.

Current quality standards for Arecae Semen mainly rely on the determination of arecoline as a single chemical marker. Although such an approach is convenient for routine testing, it cannot adequately reflect the complexity of alkaloid composition or the relationships among different constituents. The fingerprint method established in this study overcomes this limitation by simultaneously evaluating multiple alkaloid constituents and their relative distribution patterns. Consequently, it provides a more comprehensive and scientifically sound assessment of Arecae Semen quality.

Based on the similarity analysis results, a similarity threshold of not less than 0.90 is proposed as a preliminary acceptance criterion for the alkaloid fingerprint of mature Arecae Semen. This criterion effectively distinguishes mature samples from immature materials and may serve as a useful supplementary parameter for future quality standards. The integration of chromatographic fingerprinting with multi-component alkaloid characterization provides a promising strategy for improving the quality control and standardization of Arecae Semen and may support the future development of pharmacopoeia monographs for this medicinal material.

The present findings are consistent with previous studies reporting that arecoline and related alkaloids are the predominant alkaloid constituents of Arecae Semen. Similar fingerprint-based quality evaluation strategies have also been successfully applied to other traditional herbal medicines, demonstrating that chromatographic fingerprints can provide a more comprehensive assessment of chemical consistency than single-marker determination alone.

A limitation of the present study is that the sample set was collected only from Vietnam. Additional investigations involving samples from different countries and cultivation environments would be valuable for further evaluating the robustness and universality of the proposed fingerprint model.

5. CONCLUSION

A robust and reliable HPLC fingerprint method for the analysis of alkaloid constituents in Arecae Semen was successfully established and validated. The optimized chromatographic conditions enabled the generation of a

characteristic fingerprint containing six common peaks, among which four major alkaloids, namely arecoline, guvacoline, arecaidine, and guvacine, were unequivocally identified and employed as reference markers for fingerprint characterization.

The developed method demonstrated satisfactory precision, repeatability, and stability, confirming its suitability for routine quality assessment. Similarity analysis of 30 batches of Arecae Semen collected from different regions of Vietnam revealed that mature samples exhibited highly consistent alkaloid profiles, whereas immature samples showed substantial compositional variation and significantly lower similarity values. These findings indicate that harvest maturity is an important factor influencing the chemical quality of Arecae Semen.

Compared with conventional single-marker evaluation, the proposed fingerprint approach provides a more comprehensive assessment of the overall alkaloid composition and distribution patterns of Arecae Semen. The established method effectively distinguishes mature from immature materials and offers enhanced capability for evaluating batch-to-batch consistency.

Based on the similarity evaluation results, a fingerprint similarity threshold of not less than 0.90 is proposed as a preliminary quality control criterion for Arecae Semen. The present study provides a scientific foundation for the standardization and quality control of Arecae Semen and may contribute to the future development and revision of pharmacopoeial standards for this medicinal material in Vietnam and other countries.

To the best of our knowledge, this is the first study to establish an alkaloid-based chromatographic fingerprint for the quality evaluation of Vietnamese Arecae Semen using four characteristic alkaloid markers. The proposed approach provides a practical framework for transitioning from single-marker quality control to multi-component fingerprint-based evaluation.

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REFERENCES

[1] Ministry of Health of Vietnam. Vietnamese Pharmacopoeia. Hanoi: Science and Technology Publishing House; 2025. p. 1271–1272. Appendix 12.23, Guidelines for Establishing Chemical Fingerprints of Medicinal Materials by Liquid Chromatography and Gas Chromatography.

[2] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Vol. I. English Edition. Beijing: China Medical Science Press; 2020. p. 47–48. Arecae Semen; Appendix 9101.

[3] Chinese Medicine Regulatory Office. Hong Kong Chinese Materia Medica Standards. Vol. 6. Hong Kong (Monograph: Areace Semen): Department of Health, Hong Kong SAR Government; Appendix XII, Gas Chromatographic and High-Performance/ Ultra-High Performance Liquid Chromatographic Fingerprinting. Available from:
https://www.cmro.gov.hk/files/hkcmms/vol6/pdf_e/Appendix_XII_v6_e.pdf

[4] Sun H, Wang Y, Zhang Y, et al. Bioactive Components of Areca Nut: An Overview of Their Positive Impacts Targeting Different Organs. Nutrients. 2024;16(5):695. doi:10.3390/nu16050695.

[5] Chen X, Deng Y, Wang Z, et al. Chemical Composition, Pharmacological, and Toxicological Effects of Betel Nut. Evidence-Based Complementary and Alternative Medicine. 2021;2021:1808081. doi:10.1155/2021/1808081.

[6] Zou CC, Yan HY. Research Progress on Chromatographic Fingerprint Similarity Evaluation Method for Traditional Chinese Medicine in the Past 30 Years (1988–2017) and Its Prospect. Zhongguo Zhong Yao Za Zhi. 2018;43(10):1969–1977. doi:10.19540/j.cnki.cjcmm.20180115.018.

[7] Xue Y, Zhu L, Yi T. Fingerprint Analysis of Resina Draconis by Ultra-Performance Liquid Chromatography. Chemistry Central Journal. 2017;11:67. doi:10.1186/s13065-017-0299-

[8] Supplementary materials are available on the online article on the journal's website (https://tapchikiemnghiem.vn/).